# Polymorphisms in mTORC1 Genes Modulate Risk of Esophageal Squamous Cell Carcinoma in Eastern Chinese Populations

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**Introduction:** Mammalian target of rapamycin complex 1 (mTORC1) is an evolutionary conserved multiprotein complex that functions as a key regulator of gene transcription, protein translation, and autophagy. No studies have assessed associations between functional single nucleotide polymorphisms (SNPs) in mTORC1 genes and risk of esophageal squamous cell carcinoma (ESCC).

**Methods:** In a case–control study of 1126 ESCC patients and 1131 cancer-free controls, we genotyped eight SNPs in mTORC1 (*mTOR* rs1883965 G>A and rs2536 T>C, *mLST8* rs3160 C>T and rs26865 G>A, *RPTOR* rs3751934 C>A, rs1062935 T>C, rs3751932 T>C and rs12602885 G>A) and assessed their associations with risk of ESCC.

**Results:** In the single-locus analyses, we found a significantly altered risk of ESCC associated with *mTOR* rs1883965 A variant genotypes (adjusted OR = 1.27 and 1.26; 95% confidence interval = 1.01–1.60 and 1.01–1.58 for GA and GA/AA, respectively, compared with GG) but not with other SNPs. In the combined analysis of the eight SNPs, we found individuals with two or more unfavorable genotypes exhibited an increased risk for ESCC (adjusted OR = 1.35; 95% confidence interval = 1.20–1.62), compared with those with less than two unfavorable genotypes. Such a cumulative effect was dose-dependent ($p_{trend}$ = 0.004). In the multiple dimension reduction analysis, *mTOR* rs1883965 was consistently suggested as the strongest individual factor for ESCC risk, and the model including all SNPs yielded the lowest prediction error of 17.66% for model validation.

**Conclusions:** These findings suggest that functional SNPs of mTORC1 genes may individually or collectively contribute to ESCC risk. Further validation of these findings is warranted.

**Key Words:** mTORC1, Genetic polymorphisms, Risk, Esophageal cancer, Gene–environment interaction.

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Esophageal cancer has a markedly high incidence in China, compared with other geographical areas in the world. It is estimated that approximately 482,300 new esophageal cancer cases occurred in 2008 worldwide, about half of which took place in China with 90% of esophageal squamous cell carcinoma (ESCC). Previously established environmental risk factors for ESCC include tobacco use, alcohol consumption, nutrition deficiencies, and diet.[1] However, these environmental risk factors cannot readily explain overall cancer incidence, and thus hereditary factors must be involved in the etiology of ESCC. Indeed, genetic variants in a variety of candidate genes have been demonstrated to be associated with ESCC risk.[2,3]

Recently, mammalian target of rapamycin complex 1 (mTORC1) has emerged as a key regulator of numerous essential cellular processes, including translation, transcription, and autophagy,[4] and deregulation of this complex has been found to contribute to the development of cancers and the curative effect of some anticancer drugs on cancers, including ESCC.[5–8] Furthermore, the mTOR pathway was found to be aberrantly activated in most ESCC tumors, supporting a role for the mTOR activation in the etiology of ESCC.[9] The *mTOR* gene, also known as *FRAP1*, has been mapped to chromosome 1p36.2 and encodes a 300 kDa protein kinase, which emerges as a critical component of an ancient nutrient and energy effector pathway.[10] The mTOR has two distinct evolutionarily conserved complexes termed mTORC1 and mTORC2. The former is sensitive, but the latter is resistant, to the selective inhibitor rapamycin.[11] Besides mTOR, mTORC1 contains regulatory-associated protein of mTOR (RPTOR) and mLST8 (also known as GBL), both of which have a protein association.[11] The *RPTOR* and *mLST8* genes have been

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mapped to 17q25.3 and 16p13.3, respectively. The RPTOR forms a stoichiometric complex with mTOR and plays a role in nutrient-stimulated signaling to S6K1, maintenance of cell size and mTOR protein expression,[5] whereas the mLST8 can bind to the kinase domain of mTOR and regulate the mTOR kinase activity.[11]

In light of the critical role of the mTORC1 pathway in maintaining proper cellular function, it is possible that some functional single nucleotide polymorphisms (SNPs) of genes involved in this pathway may have an effect on cancer risk. Because no published studies have investigated the role of SNPs of the mTORC1 genes in the etiology of ESCC, we genotyped some selected potential functional SNPs of the mTORC1 genes and investigated their associations with the risk of ESCC in a Chinese population.

## MATERIALS AND METHODS

### Study Subjects

The study included 1126 patients with ESCC and 1131 cancer-free controls, who were all genetically unrelated ethnic Han Chinese from Eastern China. Patients recruited into this study were diagnosed with newly diagnosed and histopathologically confirmed primary ESCC from Shanghai Cancer Center, Fudan University between March 2009 and September 2011, with a response rate of 93%. Exclusion criteria included patients who had other cancers, primary tumors outside the esophagus, and tumors of an unknown origin. Cancer-free controls, frequency matched to the cases by age (±5 years) and sex, were recruited from the Taizhou Longitudinal Study at the same time period, as described previously,[12] in which the response rate was approximately 90% among those who were approached for participation in the study. Having provided a written informed consent, each participant was personally interviewed for the collection of demographic data and environmental exposure history, such as age, sex, ethnicity, body mass index (BMI), tobacco and alcohol consumption. At the end of the interview, each subject donated approximately 10-ml venous blood sample, of which 1 ml was used for genomic DNA extraction. This study was approved by the institutional review board of the Shanghai Cancer Center, Fudan University.

### SNP Selection and Genotyping

We searched the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/) for common, potentially functional SNPs and SNPinfo (http://snpinfo.niehs.nih.gov/) to identify the candidate SNPs based on the following four criteria: (1) located at the regulatory region of genes (i.e., exon, the 5′ near gene, 5′ untranslated regions [UTR], 3′UTR, 3′ near gene and splice sites); (2) the minor allele frequency (MAF) ≥ 5% in Chinese Han, Beijing descendants reported in HapMap; (3) affecting the microRNA binding sites activity or affecting transcription factor binding site activity in the putative promoter region or changing the amino acid in the exons; (4) not included in the SNP chips used in the published genome-wide association studies (GWAS) of ESCC. Only SNPs satisfied most of these criteria were finally selected. For the *RPTOR* gene, we initially identified eight SNPs (rs4890040, rs4890039, rs12602885, rs11658698, rs3751934, rs1062935, rs3751932, and rs1045626). However, because rs1045626 was in high LD with rs1062935 ($r^2$ = 0.976), we only chose rs1062935 in the 3′UTR region. In addition, we excluded three SNPs (rs11658698, rs4890040, and rs4890039) located in the 5′ near gene, because rs11658698 was included in GWAS,[13] whereas the other two SNPs were in high LD with rs11150863 ($r^2$ = 0.975, 1.000 for rs4890040 and rs4890039, respectively), which is also included in GWAS.[13] As a result, we selected three SNPs in the 3′UTR of *RPTOR* (rs3751934 C>A, rs1062935 T>C, and rs3751932 T>C) and one SNP in the 5′UTR of *RPTOR* (rs12602885 G>A) for genotyping. For the *mTOR* gene, we initially identified three SNPs (rs1883965, rs2536, and rs12125777). Because rs12125777 was in complete LD with rs2536 ($r^2$ = 1.000), we only chose rs2536 that was investigated in a previous study.[14] Finally, we included one SNP in the intron-1 region of *mTOR* (rs1883965 G>A), one SNP in the 3′UTR of *mTOR* (rs2536 T>C) for genotyping. For the *mLST8* gene, we initially confirmed three SNPs (rs26865, rs27699, and rs3160). Because rs27699 was in high LD with rs26865 ($r^2$ = 0.911), we only chose rs26865. Finally, we selected one SNP in the promoter region of *mLST8* (rs26865 G>A) and one SNP in the 3′UTR of *mLST8* (rs3160 C>T) for genotyping.

In the present study, we could not include any SNPs in exons for genotyping because no nonsynonymous SNPs met the selection criteria with MAF ≥ 5% in Chinese Han, Beijing. Genomic DNA was isolated from blood samples, and the TaqMan assay was performed for genotyping, as described previously.[15]

### Statistical Methods

The $\chi^2$ test was used to assess differences in the frequency distributions of the selected demographic variables, risk factors, and genotype of the selected SNPs between the cases and controls. Univariate and multivariate logistic regression analyses were performed to calculate crude and adjusted ORs and their 95% confidence intervals (CIs) for ESCC risk, which were also stratified by the subgroups of age, sex, BMI, and smoking and drinking status. The Hardy–Weinberg equilibrium for genotype distribution in controls was tested by a goodness-of-fit $\chi^2$ test.

To account for chance associations from multiple comparisons, we used the false-positive report probability (FPRP) to assess the false-positive association findings. We calculated FPRP with prior probabilities of 0.0001, 0.001, 0.01, 0.1, and 0.25. Results with an FPRP value less than 0.20 were considered a noteworthy association.[16] All tests were two-sided, and a $p < 0.05$ was considered statistically significant. All statistical analyses were performed with SAS software (version 9.1; SAS Institute, Cary, NC).

The possible high-order gene–gene or gene–environment interactions in the association with ESCC risk were identified by using the multiple dimension reduction (MDR) software (V2.0 beta 8.2).[17] We used 100-fold cross-validation and 1000-fold permutation testing under the null hypothesis of no association, and the best candidate interaction model was

 

selected as the one with the minimum average prediction error and the maximum cross-validation consistency (CVC).

## RESULTS

### Characteristics of the Study Population

There were no statistically significant differences in the distributions of age and sex between the cases and controls. However, the cases were more likely to be smokers (61.55% versus 54.02%; $p = 0.0003$) and drinkers (44.44% versus 32.63%; $p < 0.0001$) as well as having BMI < 25.0 (63.68% versus 43.94%; $p < 0.0001$) than the controls (Table 1). Therefore, these variables were further adjusted for in later multivariate analyses.

### Association between Eight Selected SNPs and ESCC Risk

All the observed genotype frequencies for the eight selected SNPs agreed with the Hardy–Weinberg equilibrium in the controls, and all the SNP calling rates were more than 99.00%. In the single-locus analyses, we found that significantly increased ESCC risk was associated with *mTOR* rs1883965 A variant genotypes (adjusted OR = 1.27, 95% CI = 1.01–1.60 for GA versus GG; adjusted OR = 1.26, 95% CI = 1.01–1.58 for GA/AA versus GG) and *RPTOR* rs12602885 A variant genotypes (adjusted OR = 0.68, 95% CI = 0.47–0.98 for AA versus GG). However, these risk associations were not observed for other individual SNPs (Table 2).

In the combined analysis of eight SNPs in mTORC1, we categorized all putative risk (OR > 1.0) genotypes from each SNP into a new variable according to the number of risk genotypes (for the protective genotype, OR < 1.0, we reversed the reference group). As a result, we found that individuals with two or more unfavorable genotypes exhibited increased risk for ESCC (adjusted OR = 1.35, 95% CI = 1.20–1.62), compared with those with less than two unfavorable genotypes. Furthermore, such a cumulative effect on risk was risk-genotype dose-dependent, as evidenced by a significantly increased risk of ESCC with an increasing number of observed risk genotypes (adjusted OR = 2.40, 95% CI = 1.19–4.83 for two risk genotypes; adjusted OR = 2.45, 95% CI = 1.22–4.94 for three risk genotypes; adjusted OR = 2.82, 95% CI = 1.36–5.84 for four risk genotypes; $p_{trend} = 0.004$; Table 2). In addition, we discovered that a combination of SNPs in all genes under investigation produced a higher level of risk compared with two SNPs in the same gene (Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A403).

**TABLE 1.** Distributions of Selected Variables in Esophageal Squamous Cell Carcinoma Cases and Cancer-Free Controls in an Eastern Chinese Population

| Variables | Controls, No. (%) | Cases, No. (%) | $p^a$ |
|---|---|---|---|
| All subjects | 1131 (100%) | 1126 (100%) | |
| Age, yr (mean±SD)b | 60.77 ± 10.24 | 60.40 ± 8.30 | 0.125 |
| ≤50 | 156 (13.79) | 138 (12.26) | |
| >50, ≤60 | 405 (35.81) | 422 (37.48) | |
| >60, ≤70 | 401 (35.46) | 429 (38.10) | |
| >70 | 169 (14.94) | 137 (12.17) | |
| Sex | | | 0.078 |
| Males | 879 (77.72) | 909 (80.37) | |
| Females | 252 (22.28) | 217 (19.27) | |
| Smoking status | | | 0.0003 |
| Yes | 611 (54.02) | 693 (61.55) | |
| No | 520 (45.98) | 433 (38.45) | |
| Drinking status | | | <0.0001 |
| Yes | 369 (32.63) | 501 (44.44) | |
| No | 762 (67.37) | 625 (55.56) | |
| Pack-years | | | <0.0001 |
| 0 | 520 (45.98) | 429 (38.10) | |
| ≤16 (mean) | 245 (21.66) | 152 (13.50) | |
| >16 (mean) | 366 (32.36) | 545 (48.40) | |
| Body mass index | | | <0.0001 |
| <25.0 | 497 (43.94) | 717 (63.68) | |
| ≥25.0 | 634 (56.06) | 409 (36.32) | |

[a] Two-sided $\chi^2$ test for distributions between cases and controls.
[b] Data are mean±SD and $p$ value from Student's $t$ test.

### Stratification Analysis

We further estimated the combined effect of those unfavorable genotypes on ESCC risk stratified by selected variables. As shown in Table 3, the increased risk of ESCC associated with two or more unfavorable genotypes was more pronounced among older subjects (adjusted OR= 1.66, 95% CI = 1.29–2.15), males (adjusted OR = 1.41, 95% CI = 1.15–1.74), ever-smokers (adjusted OR = 1.55, 95% CI = 1.21–1.98), never-drinkers (adjusted OR = 1.47, 95% CI = 1.16–1.86) and subjects with BMI < 25.0 (adjusted OR = 1.41, 95% CI = 1.10–1.80), compared with those with less than two unfavorable genotypes. However, further homogeneity tests showed that there were no significant differences ($p > 0.05$) among all strata except for older subjects ($p$ for homogeneity = 0.026). In addition, there was no statistical evidence of gene–environment interactions between the variant genotypes and these variables on ESCC risk.

### Association of High-Order Interactions with ESCC Risk by MDR Analysis

In the MDR analysis, we found that rs1883965 was the best one-factor model with a maximum CVC of 98 of 100 and a minimum lowest prediction error of 48.4% among all studied SNPs ($p = 0.0479$ for the prediction error). More interestingly, the eight-factor model including the combination of all studied SNPs had a minimum prediction error of 33.2% and a maximum CVC of 100 of 100, and the prediction error was statistically significant ($p < 0.0001$), which was showed to be the best model to predict ESCC risk for this study population (Table 4).

### FPRP Values for All Significant Associations

With the assumption of a moderate prior probability of 0.1 and the observed ORs, the FPRP values were 0.174, 0.150, 0.121, 0.028, and 0.004, respectively, for associations of the 2

**TABLE 2.** Genotype Frequencies of the *mTOR*, *mLST8*, *RPTOR* Polymorphisms and their Association with Risk of Esophageal Squamous Cell Carcinoma

| Variants | Cases[a], No. (%) | Controls[a], No. (%) | p[b] | Crude OR (95% CI) | p[c] | Adjusted OR (95% CI)[b] | p[d] |
|---|---|---|---|---|---|---|---|
| *mTOR* rs1883965 | | | | | | | |
| GG | 908 (80.85) | 940 (83.85) | 0.148[e] | 1.00 | | 1.00 | |
| GA | 209 (18.62) | 174 (15.53) | | 1.24 (1.00–1.55) | 0.053 | 1.27 (1.01–1.60) | 0.042 |
| AA | 6 (0.53) | 7 (0.62) | | 0.89 (0.30–2.65) | 0.831 | 1.02 (0.33–3.16) | 0.971 |
| GA/AA | 215 (19.15) | 181 (16.15) | 0.062[f] | 1.23 (0.99–1.53) | 0.063 | 1.26 (1.01–1.58) | 0.045 |
| GG/GA | 1117 (99.47) | 1114 (99.38) | | 1.00 | | 1.00 | |
| AA | 6 (0.53) | 7 (0.62) | 0.778[g] | 0.86 (0.29–2.55) | 0.779 | 0.98 (0.32–3.03) | 0.973 |
| *mTOR* rs2536 | | | | | | | |
| TT | 951 (84.68) | 957 (85.37) | 0.898[e] | 1.00 | | 1.00 | |
| TC | 165 (14.69) | 157 (14.01) | | 1.06 (0.84–1.34) | 0.642 | 1.12 (0.88–1.43) | 0.365 |
| CC | 7 (0.62) | 7 (0.62) | | 1.01 (0.35–2.88) | 0.991 | 1.21 (0.41–3.57) | 0.736 |
| TC/CC | 172 (15.32) | 164 (14.63) | 0.649[f] | 1.06 (0.84–1.33) | 0.649 | 1.12 (0.88–1.43) | 0.343 |
| TC/TT | 1116 (99.38) | 1114 (99.38) | | 1.00 | | 1.00 | |
| CC | 7 (0.62) | 7 (0.62) | 0.997[g] | 1.00 (0.35–2.86) | 1.000 | 1.19 (0.40–3.51) | 0.759 |
| *mLST8* rs3160 | | | | | | | |
| CC | 301 (26.83) | 285 (25.42) | 0.711[e] | 1.00 | | 1.00 | |
| CT | 567 (50.53) | 571 (50.94) | | 0.94 (0.77–1.15) | 0.545 | 0.93 (0.75–1.14) | 0.462 |
| TT | 254 (22.64) | 265 (23.64) | | 0.91 (0.72–1.15) | 0.421 | 0.88 (0.69–1.12) | 0.289 |
| CT/TT | 821 (73.17) | 836 (74.58) | 0.449[f] | 0.93 (0.77–1.12) | 0.450 | 0.91 (0.75–1.11) | 0.341 |
| CT/CC | 868 (77.36) | 856 (76.36) | | 1.00 | | 1.00 | |
| TT | 254 (22.64) | 265 (23.64) | 0.574[g] | 0.95 (0.78–1.15) | 0.574 | 0.92 (0.75–1.13) | 0.443 |
| *mLST8* rs26865 | | | | | | | |
| GG | 399 (35.56) | 369 (32.92) | 0.206[e] | 1.00 | | 1.00 | |
| GA | 540 (48.13) | 541 (48.26) | | 0.92 (0.77–1.11) | 0.397 | 0.90 (0.75–1.10) | 0.302 |
| AA | 183 (16.31) | 211 (18.82) | | 0.80 (0.63–1.02) | 0.076 | 0.81 (0.63–1.04) | 0.092 |
| GA/AA | 723 (64.44) | 752 (67.08) | 0.187[f] | 0.89 (0.75–1.06) | 0.187 | 0.88 (0.73–1.05) | 0.152 |
| GA/GG | 939 (83.69) | 910 (81.18) | | 1.00 | | 1.00 | |
| AA | 183 (16.31) | 211 (18.82) | 0.118[g] | 0.84 (0.68–1.05) | 0.119 | 0.85 (0.68–1.07) | 0.170 |
| *RPTOR* rs3751934 | | | | | | | |
| CC | 409 (36.55) | 430 (38.43) | 0.191[e] | 1.00 | | 1.00 | |
| CA | 529 (47.27) | 538 (48.08) | | 1.03 (0.86–1.24) | 0.719 | 1.07 (0.89–1.30) | 0.461 |
| AA | 181 (16.18) | 151 (13.49) | | 1.26 (0.98–1.63) | 0.075 | 1.27 (0.97–1.65) | 0.081 |
| CA/AA | 710 (63.45) | 689 (61.57) | 0.359[f] | 1.08 (0.91–1.29) | 0.359 | 1.12 (0.93–1.33) | 0.226 |
| CA/CC | 938 (83.82) | 968 (86.51) | | 1.00 | | 1.00 | |
| AA | 181 (16.18) | 151 (13.49) | 0.074[g] | 1.24 (0.98–1.56) | 0.075 | 1.22 (0.95–1.55) | 0.113 |
| *RPTOR* rs1062935 | | | | | | | |
| TT | 307 (27.44) | 318 (28.42) | 0.874[e] | 1.00 | | 1.00 | |
| TC | 564 (50.40) | 557 (49.78) | | 1.05 (0.86–1.28) | 0.633 | 1.08 (0.89–1.33) | 0.434 |
| CC | 248 (22.16) | 244 (21.81) | | 1.05 (0.83–1.33) | 0.670 | 1.07 (0.84–1.37) | 0.592 |
| TC/CC | 812 (72.56) | 801 (71.58) | 0.604[f] | 1.05 (0.87–1.26) | 0.604 | 1.08 (0.89–1.31) | 0.432 |
| TC/TT | 871 (77.84) | 875 (78.19) | | 1.00 | | 1.00 | |
| CC | 248 (22.16) | 244 (21.81) | 0.838[g] | 1.02 (0.84–1.25) | 0.838 | 1.02 (0.83–1.25) | 0.885 |
| *RPTOR* rs3751932 | | | | | | | |
| TT | 840 (75.07) | 846 (75.60) | 0.144[e] | 1.00 | | 1.00 | |
| TC | 250 (22.34) | 257 (22.97) | | 0.98 (0.80–1.20) | 0.840 | 0.99 (0.81–1.22) | 0.938 |
| CC | 29 (2.59) | 16 (1.43) | | 1.83 (0.98–3.39) | 0.056 | 1.83 (0.97–3.47) | 0.064 |
| TC/CC | 279 (24.93) | 273 (24.40) | 0.449[f] | 1.03 (0.85–1.25) | 0.769 | 1.04 (0.85–1.27) | 0.691 |
| TC/TT | 1090 (97.41) | 1103 (98.57) | | 1.00 | | 1.00 | |
| CC | 29 (2.59) | 16 (1.43) | 0.050[g] | 1.83 (0.99–3.40) | 0.054 | 1.83 (0.97–3.47) | 0.062 |

(*Continued*)

 

**TABLE 2.** (*Continued*)

| Variants | Cases[a], No. (%) | Controls[a], No. (%) | p[b] | Crude OR (95% CI) | p[c] | Adjusted OR (95% CI)[b] | p[d] |
|---|---|---|---|---|---|---|---|
| *RPTOR* rs12602885 | | | | | | | |
| GG | 629 (56.21) | 607 (54.24) | 0.184[e] | 1.00 | | 1.00 | |
| GA | 433 (38.70) | 435 (38.87) | | 0.96 (0.81–1.14) | 0.650 | 0.92 (0.77–1.10) | 0.347 |
| AA | 57 (5.09) | 77 (6.88) | | 0.71 (0.50–1.02) | 0.067 | 0.68 (0.47–0.98) | 0.040 |
| GA/AA | 490 (43.79) | 512 (45.76) | 0.350[f] | 0.92 (0.78–1.09) | 0.350 | 0.88 (0.74–1.05) | 0.150 |
| GA/GG | 1062 (94.91) | 1042 (93.12) | | 1.00 | | 1.00 | |
| AA | 57 (5.09) | 77 (6.88) | 0.075[g] | 0.73 (0.51–1.03) | 0.076 | 0.70 (0.49–1.01) | 0.056 |
| No. of at-risk genotypes[h] | | | | | | | |
| 0 | 13 (1.16) | 27 (2.41) | 0.047 | 1.00 | | 1.00 | |
| 1 | 324 (28.98) | 376 (33.60) | | 1.79 (0.91–3.53) | 0.093 | 1.91 (0.95–3.82) | 0.070 |
| 2 | 309 (27.64) | 291 (26.01) | | 2.21 (1.12–4.36) | 0.023 | 2.40 (1.19–4.83) | 0.014 |
| 3 | 286 (25.58) | 263 (23.50) | | 2.26 (1.14–4.47) | 0.019 | 2.45 (1.22–4.94) | 0.012 |
| 4 | 137 (12.25) | 114 (10.19) | | 2.50 (1.23–5.06) | 0.011 | 2.82 (1.36–5.84) | 0.005 |
| 5 | 43 (3.85) | 40 (3.57) | | 2.23 (1.01–4.92) | 0.046 | 2.42 (1.07–5.43) | 0.033 |
| 6 | 6 (0.54) | 8 (0.71) | | 1.56 (0.45–5.43) | 0.487 | 1.79 (0.49–6.56) | 0.383 |
| Trend | | | | | 0.008 | | 0.004 |
| Dichotomized groups | | | | | | | |
| 0–1 | 337 (30.14) | 403 (36.01) | 0.003 | 1.00 | | 1.00 | |
| 2–6 | 781 (69.86) | 716 (63.99) | | 1.30 (1.09–1.56) | 0.003 | 1.35 (1.20–1.62) | 0.002 |

[a] The numbers of each single nucleotide polymorphism were less than the total number of subjects because some genotyping data were unavailable.
[b] Two sides $\chi^2$ test for genotype distributions between cases and controls.
[c] Unadjusted for age, sex, BMI, smoking status, and drinking status in logistic regression models.
[d] Adjusted for age, sex, BMI, smoking status, and drinking status in logistic regression models.
[e] For additive genetic models.
[f] For dominant genetic models.
[g] For recessive genetic models.
[h] The risk genotypes used for the calculation were *mTOR* rs1883965 GA/AA + rs2536 TC/CC, *mLST8* rs3160 CC + rs26865 GG, *RPTOR* rs3751934 AA + rs1062935 CC + rs3751932 CC + rs12602885 GA+GG.
CI, confidence interval; BMI, body mass index.

to 6 combined risk genotypes with an increased risk of ESCC in all subjects and in subgroups of older subjects. The FPRPs for all of the above-mentioned significant associations were below the threshold of 0.20, suggesting that these associations were noteworthy findings as presented in the current study (Table 5).

## DISCUSSION

In the present study, we found that *mTOR* rs1883965 A variant genotypes were individually associated with an increased ESCC risk in the Eastern Chinese population. When all unfavorable genotypes were combined, risk of ESCC increased as the number of at-risk genotypes increased, suggesting that these eight SNPs may collectively contribute to risk of ESCC. Furthermore, results from the MDR analysis also consistently identified *mTOR* rs1883965 as the main single susceptibility locus and the combinations of all studies SNPs contributed to ESCC risk. These findings seem to be biologically plausible.

It is known that the mTOR protein is involved in a complex signaling network of controlling cell growth, proliferation, cell cycle, and autophagy,[11] and the knockdown of mTOR in mice is embryonically lethal.[18] In addition, RPTOR interacts with mTOR to make the phosphorylation reaction of S6K1 and 4EBP1 possible, the two well-characterized mTORC1 effectors.[19] Similar to the knockout of mTOR, the knockout of RPTOR is also early embryonically lethal,[20] which underscores the biological importance of this gene. A previous study found that the knockdown of mLST8 in cells may suppress the phosphorylation of S6K1 and 4EBP1.[19]

To date, only two reported studies have investigated the association between *mTOR* polymorphisms and risk of cancers. One earlier U.S. study of 1574 colon cancer cases and 1940 healthy controls found that one tagSNP rs1057079 was associated with a significantly increased colon cancer risk.[21] The later study conducted in Chinese children with 417 acute lymphoblastic leukemia cases and 554 controls showed a protective effect of rs2536 on risk of acute lymphoblastic leukemia,[14] which is not in agreement with our findings that no association was observed for the rs2536 variant with ESCC risk. Recently, an American study of 803 bladder cancer cases and 803 controls showed that *RPTOR* gene polymorphisms, including rs1062935, were associated with an increased cancer risk,[22] which is not consistent with our null result for rs1062935. Taken together, the inconsistent results between the present study and those studies with similar SNPs but different cancers by others may be due to different genetic backgrounds or etiologies of various cancers. For example, the *mTOR* rs2536 C allele frequency of the controls in the above-mentioned Chinese study and ours was similar (0.098 versus 0.076), suggesting that there was no methodological

**TABLE 3.** Stratification Analysis for Associations between Combined Risk Genotypes of *mTOR*, *mLST8*, and *RPTOR* Polymorphisms and Esophageal Squamous Cell Carcinoma Risk

| Variables | Combined Effect of Risk Genotypes (Cases/Controls, *n*, %) | | Crude OR (95% CI) | *p* | Adjusted OR[a] (95% CI) | $p^a$ | $p^b$ | $p^c$ |
|---|---|---|---|---|---|---|---|---|
| | 0–1 At-Risk Genotype | 2–6 At-Risk Genotype | | | | | | |
| Age, yr (median) | | | | | | | | |
| ≤60 | 173/180 (31.06/32.37) | 384/376 (68.94/67.63) | 1.06 (0.83–1.37) | 0.638 | 1.08 (0.83–1.41) | 0.587 | 0.026 | 0.235 |
| >60 | 164/223 (29.23/39.61) | 397/340 (70.77/60.39) | 1.59 (1.24–2.04) | 0.000 | 1.66 (1.29–2.15) | 0.000 | | |
| Sex | | | | | | | | |
| Males | 268/319 (29.65/36.67) | 636/551 (70.35/63.33) | 1.37 (1.13–1.68) | 0.002 | 1.41 (1.15–1.74) | 0.001 | 0.261 | 0.286 |
| Females | 69/84 (32.24/33.73) | 145/165 (67.76/66.27) | 1.07 (0.73–1.58) | 0.734 | 1.04 (0.69–1.57) | 0.861 | | |
| Smoking status | | | | | | | | |
| Never | 136/172 (31.63/33.46) | 294/342 (68.37/66.54) | 1.09 (0.83–1.43) | 0.550 | 1.11 (0.84–1.47) | 0.472 | 0.081 | 0.075 |
| Ever | 201/231 (29.22/38.18) | 487/374 (70.78/61.82) | 1.50 (1.19–1.89) | 0.001 | 1.55 (1.21–1.98) | 0.001 | | |
| Drinking status | | | | | | | | |
| Never | 180/279 (28.99/37.00) | 441/475 (71.01/63.00) | 1.44 (1.15–1.81) | 0.002 | 1.47 (1.16–1.86) | 0.001 | 0.183 | 0.230 |
| Ever | 156/124 (31.45/33.97) | 340/241 (68.55/66.03) | 1.12 (0.84–1.50) | 0.435 | 1.12 (0.83–1.52) | 0.452 | | |
| BMI | | | | | | | | |
| <25.0 | 219/189 (30.72/38.34) | 494/304 (69.28/61.66) | 1.40 (1.10–1.79) | 0.006 | 1.41 (1.10–1.80) | 0.006 | 0.442 | 0.358 |
| ≥25.0 | 118/214 (29.14/34.19) | 287/412 (70.86/65.81) | 1.26 (0.96–1.66) | 0.087 | 1.25 (0.95–1.64) | 0.108 | | |

[a] Obtained in logistic regression models with adjustment for age, sex, BMI, smoking status, and drinking status.
[b] *p* for homogeneity test using the $\chi^2$-based *Q* test.
[c] Test for multiplicative interaction obtained from logistic regression models with adjustment for age, sex, BMI, smoking status, and drinking status.
CI, confidence interval; BMI, body mass index.

bias in the genotyping method. Further validations in additional larger studies of different cancer types either in additional group of homogenous ethnicity or in more ethnically diverse groups are warranted.

The present study, for the first time, found that SNP rs1883965 located in the intron-1 region of *mTOR* was associated with increased ESCC risk. Several studies have discovered that some disease-associated *functional* intronic variants may alter mRNA levels of genes by affecting the transcriptional efficiency, RNA elongation, or splicing.[23–25] For rs1883965, TRANSFA (http://www.gene-regulation.com) has predicted that it may affect the transcription factor binding site activity. Therefore, we speculated that it may be a causal SNP or in LD with either other functional polymorphisms, thereby altering the function of *mTOR* or with SNPs of an adjacent susceptibility gene. In fact, further LD analysis using the SNPinfo database found that rs1883965 was in high LD with a synonymous SNP rs1064261 ($r^2$ = 0.864), which was predicted to have an effect on splicing. It should be noted that the results derived from single SNP analysis tend to have a high false discovery rate, because multiple hypotheses are tested simultaneously and that the probability of type I error rates inflates with the increasing number of tests. Indeed, in the present study, greater FPRP values observed for the significant associations of rs1883965 variants with ESCC risk suggested a possible false-positive finding. However, all methods of testing multiple comparisons are very conservative when exploring candidate genes with *a prior* hypothesis. In any rate, additional large studies are required to replicate our findings.

We noticed that effect of the combined unfavorable genotypes on ESCC risk was more pronounced among older subjects. It is likely that older subjects may have been exposed environmental carcinogens for a longer time, which possibly led to more DNA damage that may have interacted with genetic variations in initiating carcinogenesis. However, we observed neither associations for other subgroups nor any statistical evidence for gene–environment interactions between the variant genotypes and these variables on risk of ESCC. The possible reason may be that this subgroup finding often suffers from reduced statistical power or simply due to chances. Therefore, such a finding needs to be confirmed in future larger studies.

**TABLE 4.** Multiple Dimension Reduction Analysis for the Esophageal Squamous Cell Carcinoma Risk Predication

| Best Model | Cross-Validation | Avg. Prediction Error | $p^a$ |
|---|---|---|---|
| 1 | 98/100 | 0.484 | 0.0479 |
| 2, 3 | 98/100 | 0.466 | 0.0002 |
| 2, 3, 4 | 100/100 | 0.448 | <0.0001 |
| 2, 3, 4, 5 | 75/100 | 0.436 | <0.0001 |
| 1, 2, 3, 4, 6 | 100/100 | 0.414 | <0.0001 |
| 1, 2, 3, 4, 6, 7 | 100/100 | 0.389 | <0.0001 |
| 1, 2, 3, 4, 6, 7, 8 | 86/100 | 0.362 | <0.0001 |
| 1, 2, 3, 4, 5, 6, 7, 8[b] | 100/100 | 0.332 | <0.0001 |

[a] *p* value for 1,000-fold permutation test.
[b] The best model was selected as the one with the minimum average prediction error and maximum cross-validation. In this study, the best interaction model is indicated in boldface.
Labels: 1: rs1883965, 2: rs3751934, 3: rs1062935, 4: rs26865, 5: rs2536, 6: rs12602885, 7: rs3160, and 8: rs3751932.

 

**TABLE 5.** False-Positive Report Probability Values for Associations between Esophageal Squamous Cell Carcinoma Risk and Genotypes of *mTOR, mLST8,* and *RPTOR* Polymorphisms

| Genotype | Positive OR (95% CI)[a] | $p^b$ | Statistical Power[c] | Prior Probability 0.25 | 0.10 | 0.01 | 0.001 | 0.0001 |
|---|---|---|---|---|---|---|---|---|
| *mTOR* rs1883965 | | | | | | | | |
| GA vs. GG | 1.24 (1.00–1.55) | 0.053 | 0.962 | 0.142 | 0.331 | 0.845 | 0.982 | 0.998 |
| GA/AA vs. GG | 1.23 (0.99–1.53) | 0.063 | 0.972 | 0.163 | 0.368 | 0.865 | 0.985 | 0.998 |
| *RPTOR* rs12602885 | | | | | | | | |
| AA vs. GG | 0.71 (0.50–1.02) | 0.067 | 0.640 | 0.239 | 0.485 | 0.912 | 0.991 | 0.999 |
| Combined risk genotypes | | | | | | | | |
| No. at-risk genotypes[d] | | | | | | | | |
| 2 vs. 0 | 2.21 (1.12–4.36) | 0.023 | 0.983 | 0.066 | 0.174 | 0.698 | 0.959 | 0.996 |
| 3 vs. 0 | 2.26 (1.14–4.47) | 0.019 | 0.973 | 0.055 | 0.150 | 0.659 | 0.951 | 0.995 |
| 4 vs. 0 | 2.50 (1.23–5.06) | 0.011 | 0.721 | 0.044 | 0.121 | 0.602 | 0.938 | 0.993 |
| 5 vs. 0 | 2.23 (1.01–4.92) | 0.046 | 0.481 | 0.223 | 0.463 | 0.904 | 0.99 | 0.999 |
| 2–6 vs. 0–1 | 1.30 (1.09–1.56) | 0.003 | 0.930 | 0.010 | 0.028 | 0.242 | 0.763 | 0.970 |

[a] The crude OR reported in Tables 2 and 3.
[b] Calculated the genotype frequency distributions using the omnibus $\chi^2$ test in Tables 2 and 3.
[c] Calculated statistical power using the number of observations and the OR and $p$ values in Tables 2 and 3.

Some significant findings of the present study suggested some complex gene–gene interactions, which were consistently identified through different analytic approaches. In the logistic regression model, a locus dose–response was found for the increased ESCC risk with the increasing number of risk or unfavorable genotypes of all studied SNPs. In the MDR analysis, eight-factor model, including the combination of all studied SNPs, is the best model to predict ESCC risk in this study population. These results suggest that all studied SNPs may have a joint effect on risk of ESCC, although the evidence of true interactions was weak and the mechanisms underlying these high interactions remain to be elucidated. Given their integration of functions of the multiprotein complex consisting of mTOR, RPTOR, and mLST8 in the mTORC1 pathway, it is biologically plausible for these potentially functional polymorphisms to have a synergistic effect that was detected in this study.

In summary, the present study investigated the associations between mTORC1 polymorphisms and ESCC risk with a relatively large sample size. One strength is that we used diverse approaches to analyze the data, including logistic regression and MDR, for accessing possible interactions. However, there are some inherited limitations. First, as a hospital-based case–control study, this study may be subject to inherent biases for selection of the nonrepresentative subjects and retrospective collection of exposure data. Second, the selection of SNPs was based on prior knowledge of potentially functional SNPs instead of tagging SNPs, which may miss some important variations within the genes. Finally, because the MAF of some SNPs in the present study was low, we had limited statistical power to detect significant associations in some subgroups, let alone the power to assess gene–environment interactions adequately. Therefore, additional larger, well-designed, and population-based studies are warranted to confirm our findings.

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